UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: **January 21, 2010**

SUBJECT: Request for Tolerance Exemption and Science Review of Prenatal Developmental Toxicology for Homobrassinolide

Decision Number: 381556
DP Number: 368544
EPA File Symbol Number: 69361-RT
Chemical Class: Biochemical
PC Code: 067700
CAS Number:80483-89-2
Active Ingredient Tolerance Exemptions:
MRID Numbers: 47817702

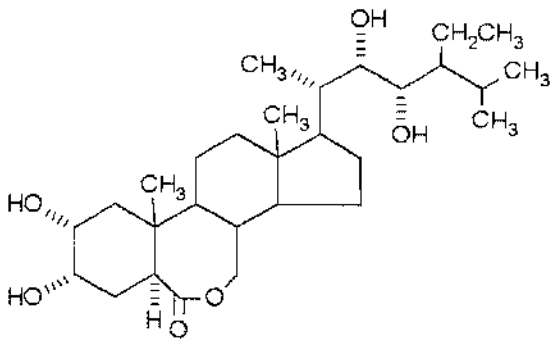
FROM: Miachel Rexrode Ph.D., Senior Biologist /s/ 01/21/10
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: John Fournier, Regulatory Action Leader /s/ 01/21/10
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

ACTION REQUESTED

1) The registrant has submitted a Prenatal Developmental Toxicology study OPPTS 870.3700 to fulfill data requirements that were outlined in a meeting between Repar Corporation and the Agency (06/18/09).

2) Repar Corporation (Repar) has also submitted a tolerance exemption petition for the active ingredient, homobrassinolide(2α,3α,22S,23S,24S)-2,3,22,23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one), on all raw agricultural commodities (food and non-food crops including forage crops and animal feed as well as residues of homobrassinolide in meat, milk, and eggs). Brassinosteroids are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom. These compounds, when applied to plants, improve their quality and yield and have been further researched for stress-protective properties (i.e. cold, heat, salt, and heavy metal exposure). Homobrassinolide is a plant growth regulator that is proposed as controlling or regulating the growth and development of all higher and lower plants. The proposed uses include all agronomic and horticultural crops such as agricultural crops, greenhouse food uses and non-food crops, aquatic food and non-food crops (all crops, grasses, vines, and trees listed under 40 CFR Part 158, Appendix A.



RECOMMENDATIONS AND CONCLUSIONS

STUDY SUMMARIES

Toxicology

MRID 47817702: Teratogenic Evaluation (OPPTS 870.3700). Data from this study showed that treatment with 100 and 1000 mg/kg bw of technical homobrassinolide (HBR) did not result in mortality or overt signs of toxicity for pregnant rats during the observation period. Body weight changes in the groups of test substance treated dams were statistically similar to controls and no significant changes were observed in the weights of ovary and fetuses. The test further showed that there were no significant changes in the uterine weights, as well as, no test related recurrent visceral and skeletal malformations when compared to controls. Based on these findings it appears that HBR

was non-teratogenic to Wistar rats at the dose levels of 100 and 1000 mg/kg bw (refer to Appendix A for statistical analysis). This study is **Acceptable.**

Product Chemistry (MRID 47185101 – 47185117)

This compound is stable for 14 days at 54±2^0 C and 62% humidity and non flammable at temperatures up to 100^0 C. The melting range is 116 – 118^0 C. The partition coefficient is log P_{ow} = 3.96 suggesting moderate lipofilicity. Solubility in water is 3.18%, acetone is 95.89% and in ethanol 99.89%. Registrant should provide the MSDSs or specification sheets for the beginning materials are provided for the listed suppliers and 2) the waivers for the one-year studies of storage stability and corrosion characteristics are granted. A PC code was assigned for homobrassinolide (PC 067700). Refer to Table 1.0.

Table 1. Physical and Chemical Properties for Homobrassinolide Technical

Guideline Reference No./Property	Description of Result	Methods
830.6303 Color	White to pale yellow	Visual Observation
830.6303 Physical State	Powder	Visual Observation
830.6304 Odor	Mild, characteristic odor (1% suspension in ultrapure water)	Olfactory inspection
830.6313 Stability	Stable for 14 days at 54±2^0 C and 62% relative humidity. The product is packaged in HDPE containers with LDPE liners, and is not likely to contact metals during its lifetime or use.	CIPAC MT 46
830.6314 Oxidation/Reduction Chemical Incompatibility	Not applicable, product is not intended to contact strong oxidizing or reducing agents.	
830.6315 Flammability	Not flammable at temperatures up to 100^0 C	OPPTS 6315 Closed Cup
830.6316 Explodability	Product has no potential to explode	
830.6317 Storage Stability	Stable for 14 days at 54±2^0 C and 62% relative humidity.	CIPAC MT 46
830.6319 Miscibility	Not applicable, product is not to be mixed with petroleum solvents.	
830.6320 Corrosion	Calculated corrosion rates based	ASTM G31-72 & D1384-87

Guideline Reference No./Property	Description of Result	Methods
Characteristics	on mean weight loss of coupons exposed to the product for 14 days: Copper - 0.00142 mm/year Aluminum - 0.0540 mm/year.	
830.6321 Dielectric Breakdown Voltage	Not required for TGAI/MP	
830.7000 pH	7.65 (1g in 100 ml of water)	CIPAC MT 75.1 Digital pH meter
830.7050 UV/Visible Absorption	^max = 1.2458 at 202 nm (10 mg in 50 ml methanol-HCL)	OECD 101
830.7100 Viscosity	Not applicable, product is a solid	
830.7220 Melting Range	116-118^0 C	OECD 102
830.7220 Boiling Range	Not applicable, product is a solid	
830.7300 Density/Relative density/Bulk Density	Density 0.7 g/ml at ambient temperature	OECD 109 Pycnometer
830.7550 Partition Coefficient	Log P_{ow} = 3.96	OECD 117
830. 7370 Dissociation Constant in Water	pKa = 6.44 at pH 6.0 6.29 at pH 6.5 6.54 at pH 7.0 7.56 at pH 7.5 7.81 at pH 8.0	OECD 112
830.7840 Water Solubility	3.18% in water 95.89% in acetone 99.89% in ethanol	CIPAC MT 157.1 & 157.2

Tier I Toxicology Waiver Requests

MRID 47185131: Waiver Request for Hypersensitivity Incidents. None of the test animals demonstrated any immediate or delayed reaction manifested by skin rash, shortness of breath, generalized swelling, and/or tearing. It is therefore unlikely that Homobrassinolide Technical would cause any allergenic reactions leading to immediate or delayed hypersensitivity. As a result, the registrant does not believe that precautionary labeling regarding eye and skin exposure is needed for Homobrassinolide Technical or end use products containing the active ingredient. Additionally, virtually no human exposure is expected after products containing Homobrassinolide are applied to target crops. This information appears sufficient to support the requested waiver for hypersensitivity incidents, although any future incidents must still be reported to the Agency per 40 CFR 158.690(c). The information submitted **is sufficient to support the requested waiver** for hypersensitivity incidents, although any future incidents must still be reported to the Agency per 40 CFR 158.690(c). This **waiver is Acceptable.**

MRID 47185132: Waiver Request for *in vitro* Mammalian Cell Gene Mutation Testing (OPPTS 870.5300) Based on the results of these three studies, MRID 47208905, 47185127, 47208904, the registrant concluded that Homobrassinolide Technical has no potential for genotoxicity. The information submitted is sufficient to support the requested waiver for *in vitro* mammalian cell gene mutation testing. **Waiver is Acceptable.**

MRID 47185133: Waiver Request for Unscheduled DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550). A test for unscheduled DNA synthesis in mammalian cells in culture is not required, and a **waiver is therefore not needed**.

MRID 47185134: Waiver Request for in vitro Mammalian Chromosomal Aberration Test (OPPTS 870.5375). The information submitted is sufficient to support the requested waiver an *in vitro* mammalian chromosomal aberration test. This **waiver is Acceptable**

MRID 47185135: Waiver Request for Immunotoxicity (OPPTS 880.3550). In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1,000 mg/kg/day. There were no changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function. This submitted information **is sufficient to support the requested waiver.** This **waiver is Acceptable.**

MRID 47185136: Waiver Request for 90-Day Dermal Toxicity (OPPTS 870.3250). Since the 90-day oral toxicity study was found to be acceptable the information submitted is sufficient to support the requested waiver for 90-day dermal testing. The active ingredient should not present any purposeful application or prolonged exposure to human skin and is not expected to be metabolized differently after dermal exposure. **This waiver is Acceptable.**

MRID 47185137: Waiver Request for 90-Day Inhalation Toxicity (OPPTS 870.3465). The ORNL reviewer for the 90-day oral toxicity study found it to be **Acceptable**. Based on the expected use patterns, there is no likelihood of significant inhalation levels from exposure to the pesticide as a gas, vapor, or aerosol. Therefore, the information submitted is sufficient to **support the requested waiver for 90-day inhalation testing**.

MRID 47185139: Waiver Request for Mammalian Mutagenicity Testing (OPP 152-19). A test for mammalian mutagenicity is not required because it was not triggered by any Tier I study conclusions. Therefore, a **waiver is therefore not needed**.

MRID 47185140: Waiver Request for Immune Response (OPPTS 880.3800).). In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1,000 mg/kg/day. There were no changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function. This submitted information **is sufficient to support the requested waiver for immune response testing.** This **waiver is Acceptable.**

MRID 47185141: Waiver Request for Chronic Exposure (OPPTS 870.4100). The acute 90-Day Oral Toxicity Gavage is acceptable. Therefore the Agency can grant a waiver for this chronic exposure study. **Waiver is Acceptable.**

MRID 47185142: Waiver Request for Carcinogenicity (OPPTS 870.4200). This compound (active ingredient or degradates) does not cause any morphologic effect (e.g. hyperplasia or metaplasia) in any organ that could lead to neoplastic change. Therefore, a carcinogenicity test is not required, and a **waiver is therefore not needed.**

Tier I Non-Target Organism Testing

MRID 47185129: Homobrassinolide Technical. Biochemical Pesticides Nontarget Organisms Toxicology Data. Acute Immobilization Test to Freshwater Fish, *Poecilia reticulata* and *Brachydanio rerio*. 96 Hr LC_{50}=24.56 mg/L (20.44-28.68 mg/L) 96 hr LC_{50}=14.38 mg/L (12.79-15.98 mg/L). This study was conducted as a static renewal and is **Acceptable**.

MRID: 47185130: Homobrassinolide Technical. Biochemical Pesticides Nontarget Organisms Toxicology Data. Acute Immobilization Test in *Daphnia magna*. 48-hr EC_{50} = 8.90 mg/L (8.47 to 9.34 mg/L. This study was conducted as a static renewal and is **Acceptable.**

Non-Target Organism Waiver Request

MRID 47185143: Waiver Request for Avian Acute Oral Toxicity (OPPTS 850.2100). The registrant calculated the anticipated application rate of homobrassinolide as follows: 20 g/A – 20,000 mg/43,560 ft^2 = 0.459 mg/ft^2. Homobrassiolide do not appear to be toxic to vertebrates. Toxicity studies on mammals show practically no toxicity (i.e. rat LD_{50} > 5,000 mg/kg; mice LD_{50} > 5, 000 mg/kg; 90-day rat oral was 1,000 mg/kg). In addition, avian species have been environmentally exposed to the active ingredient since homobrassinolides are found in all plants. The information submitted is sufficient to support the requested waiver for Avian Acute Oral Toxicity testing. This **waiver is Acceptable.**

MRID 47185144: **Waiver Request** for Avian Acute Dietary Toxicity (OPPTS 850.2200) The information submitted is sufficient to support the requested waiver for Avian Acute Dietary Toxicity testing. Homobrassiolide do not appear to be toxic to vertebrates. Toxicity studies on mammals show practically no toxicity (i.e. rat LD_{50} > 5,000 mg/kg; mice LD_{50} > 5, 000 mg/kg; 90-day rat oral was 1,000 mg/kg). In addition, avian species have been environmentally exposed to the active ingredient since homobrassinolides are found in all plants. Application of the product in the environment is expected to be low at 20 g/A with potential exposure to avian species at less than 1 mg/ft^2. The compound is to be sprayed and not applied as a granular, thus decreasing exposure. This **waiver request is Acceptable**.

MRID 47185145: Waiver Request for Nontarget Plant Studies (OPPTS 850.4000). Since, homobrassinolide occurs naturally in plants, it is unlikely that exogenous application can cause any adverse effects to nontarget plants. In addition, there does not appear to be any reported incidents of toxicity to plants. The expected application rate of homobrassinolide as an end use product is very low at 20 g/A (1 mg ft^2). Therefore, the information submitted is sufficient to support the requested waiver for nontarget plant studies. This **waiver request is Acceptable.**

MRID 47185146: Waiver Request for Nontarget Insect Testing (OPP 154-11). This compound does not exhibit any insecticidal activity. Since homobrassinosteroids and brassinolide are assumed to be ubiquitous in all plants and plant products (i.e. pollen) insects have always been exposed to these compounds without displaying toxic effects. The information submitted is sufficient to support the requested waiver for nontarget insect testing. This **waiver is Acceptable**.

Table 2. Waiver Requests for Homobrassinolide Technical Registration

Study	Rational	Agency Response	MRID
Human Toxicology			
Hypersensitivity Incidents (OPP 152-16).	Information is sufficient	**Waver Request Acceptable**	**47185131**
in vitro Mammalian Cell Gene Mutation Testing (OPPTS 870.5300)	No potential for genotoxicity	**Waver Request Acceptable**	**47185132**
DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550)	Unscheduled DNA synthesis in mammalian cells in culture is not required	**Waiver is not needed**	**47185133**
in vitro Mammalian Chromosomal Aberration Test (OPPTS 870.5375)	Information submitted is sufficient	**Waver Request Acceptable**	**47185134**
Immunotoxicity (OPPTS 880.3550)	No changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function (MRID 47208906).	**Waver Request Acceptable**	**47185135**
90-Day Dermal Toxicity (OPPTS 870.3250)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185136**
90-Day Inhalation Toxicity	Sufficient information		

Study	Rational	Agency Response	MRID
(OPPTS 870.3465)	has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185137**
Mammalian Mutagenicity Testing (OPP 152-19)	A test for mammalian mutagenicity is not required.	**Waiver is not needed**	**47185139**
Immune Response (OPPTS 880.3800)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185140**
Chronic Exposure (OPPTS 870.4100)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185141**
Carcinogenicity (OPPTS 870.4200)	A carcinogenicity test is not required	**Waiver is not needed.**	**47185142**
Ecotoxicity			
Avian Acute Oral Toxicity (OPPTS 850.2100)	Information submitted is sufficient to support the requested waiver	**Waver Request Acceptable**	**47185143**
Avian Acute Dietary Toxicity (OPPTS 850.2200)	Information submitted is sufficient to support the requested waiver	**Waver Request Acceptable**	**47185144**
Nontarget Plant Studies (OPPTS 850.4000)	Information submitted is sufficient to support the requested waiver	**Waver Request Acceptable**	**47185145**
Nontarget Insect Teesting (OPP 154-11)	Information submitted is sufficient to support the requested waiver	**Waver Request Acceptable**	**47185146**

Toxicological Profile for Homobrassinolide

Toxicological Profile for Homobrassinolide is presented in Table 3.0. This data suggests that the compound is practically non-toxic (Tox. Category III and IV). This compound should not cause chromosomal aberrations or cytotoxicity and is not a sensitizer or a dermal irritant. Exposure to eyes results in a mild irritation. Ecotoxicity data shows slightly toxic to freshwater fish (14.38 – 24.56 mg/L) and moderately toxic to aquatic invertebrates (8.90 mg/L).

Table 3.0 Toxicological Profile for Homobrassinolide Technical

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
Human Toxicity			
Acute Oral Toxicity – Mice (OPPTS 870.1100)	> 5,000 mg/L	IV	**47208903**
Mammalian Bone Marrow	No chromosome aberrations in		

Chromosomal Aberration Test (OPPTS 870.5385)	mice treated up to a single oral dose of 2000 mg/kg body weight. **Acceptable**	**No Chromosomal Aberrations**	**47208905**
SubacuteOral Toxicity-rat (OPPTS 870.3100)	> 1,000 mg/L **Acceptable**	**III**	**47208906**
Acute Oral Toxicity (OPPTS 870.1100)	> 5,000 mg/kg **Acceptable**	**IV**	**47185118**
Acute Dermal Toxicity (OPPTS 870.1200)	> 2,000 mg/kg **Acceptable**	**III**	**47185120**
Acute Inhalation Toxicity (OPPTS 870.1300)	>2.26 mg/L **Acceptable**	**IV**	**47185121**
Acute Eye Irritation (OPPTS 870.2400)	Mild irritation **Acceptable**	**III**	**47185122**
Acute Dermal Irritation (OPPTS 870.2500)	Not an irritant **Acceptable**	**IV**	**47185123**
Skin Sensitization (OPPTS 870.2600)	Not a sensitizer **Acceptable**	**IV**	**47185124**
In vivo Mammalian Cytogenetics-Erythrocyte Micronucleus assay (OPPTS 870.5395)	No a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment dose (2000 mg/kg). **Acceptable**	**Not Cytotoxic**	**47185127**
Teratogenicity (OPPTS 870.3700)	No toxicity or overt clinical signs of toxicity. This compound was non-teratogenic to Wistar rats at the dose level of 1000 mg/kg b.w. **Acceptable.**	**Not Teratogenic**	**47817702**
Bacterial Reverse Mutation Assay (OPPTS 5100)	Five dosage levels ranging from 39.06 - 625 ug/plate for strains TA100, TA1535, TA98, and 9.77 - 156.25 ug/plate for strains TA102 and TA1537 with and without S9. Testing showed that there was no concentration related or reproducible increase in the number of revertant colonies in the concentrations tested and no observable statistically significant dose-response relationship. This resubmitted test is considered **Acceptable.**	**No mutagenic effect** in the *Salmonella typhimurium* strains tested.	**47546502**

Ecotoxicity			
Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)	96 Hr LC_{50} =24.56 mg/L (20.44-28.68 mg/L) **Acceptable** 96 hr LC_{50} =14.38 mg/L (12.79-15.98 mg/L) **Acceptable**	**Slightly Toxic**	**47185129**
Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)	48-hr EC_{50} = 8.90 mg/L (8.47 to 9.34 mg/L.. **Acceptable**	**Moderate Toxicity**	**47185130**

Tier I Toxicity Studies (MRID 47208903 – 47185106, 47185118, 47185120 – 47185124, 47185127, 47208907,)

MRID 47208903: Acute Oral Toxicity - Mice (OPPTS 870.1100). The mouse oral LD_{50} for male, female, and combined was greater than 5000 mg/kg.
Acceptable Tox Catogory IV.

MRID 47208904: Previous Study Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. **Unacceptable.** The doses that were tested were extremely low: the highest dose was 10^4 times lower than the limit dose of 5,000 µg/plate recommended by the Guidelines. There was no mention of toxicity caused by the test substance, with or without activation in any tester strain. However, the study is deficient and unacceptable because the researchers used a range of low doses (0.03 to 0.5 µg/plate) ignoring higher dose ranges.. In addition, only 4 of the 5 recommended strains were tested. Almost no information was provided on the S9 fraction used for activation. The number of bacteria plated for each treatment group was not presented, making it impossible to quantify the number of revertants/10^5 viable bacterial cells. No historical control data were presented (**refer to MRID 47546502 below**).

MRID 47546502: Resubmission Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. After range finding testing with technical homobrassinolide (87.1% ai), researchers chose five dosage levels ranging from 39.06 - 625 ug/plate for strains TA100, TA1535, TA98, and 9.77 - 156.25 ug/plate for strains TA102 and TA1537 with and without S9. Testing appeared to have been conducted according to standard protocol. Results show that there was no concentration related or reproducible increase in the number of revertant colonies in the concentrations tested and no observable statistically significant dose-response relationship. The test substance, homobrassinolide, did not induce any apparent mutagenic effect in the *Salmonella typhimurium* strains tested. This resubmitted test is considered **Acceptable.**

MRID 47208905. *In Vivo* Mammalian Cytogenetics - Erythrocyte chromosomal aberration assay in mice (OPPTS 870.5385). In a chromosomal aberration test, Homobrassinolide Technical did not have the potential to induce chromosome aberrations in mice treated up to a single oral dose of 2000 mg/kg body weight. **Acceptable** and in general satisfies the guideline requirement for Test Guideline (OPPTS 870.5385) for *in vivo* cytogenetic mutagenicity data.

MRID 47208906 : 90-Day Oral Toxicity Gavage - Rat; (OPPTS 870.3100). This 90-day oral toxicity study with recovery in the Wistar rat is upgraded to **Acceptable:** The NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. There were no changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function.

MRID 47185120: Acute Dermal Toxicity - Rats (OPPTS 870.1200). The dermal LD_{50} for males, females, and combined was greater than 2000 mg/kg. **Acceptable: Tox category III.**

MRID 4785121: Acute Inhalation Toxicity - Rats (OPPTS 870.1300). The inhalation LC_{50} for males, females, and combined was > 2.26 mg/L. **Acceptable. Tox. Category IV.**

MRID 47185122: Acute Eye Irritation - Rabbits (OPPTS 870.2400). Corneal opacity was noted on 6/6 rabbits at one hour after test material instillation with resolution by day 7. Iritis was noted on 6/6 rabbits 24 hours after test material instillation with resolution by day 5. Positive conjunctival irritation (score 2 or 3) was noted on 1/6, 6/6, and 6/6 rabbits 1, 24, and 48 hours after test material instillation with resolution by 72 hours. The maximum average score was 41.33 at 24 hours after test material instillation. Homobrassinolide Technical was moderately irritating. **Acceptable. Tox. Category III.**

MRID 47185123: Primary Dermal Irritation - Rabbits (OPPTS 870.2500). No dermal irritation was noted on any rabbit. The primary irritation index was 0.0. Homobrassinolide Technical was non-irritating. **Acceptable. Tox. Category IV.**

MRID 47185124: Previous Study Skin Sensitization - Guinea Pigs (OPPTS 870.2600). **Unacceptable, but upgradeable** if the registrant provides a positive control study which was carried out within six months of the study and the results are appropriate **(refer to MRID 475446501 below).**

MRID 475446501: Resubmission Skin Sensitization - Guinea Pigs (OPPTS 870.2600). None of the animals of treatment groups and control groups presented any skin irritation at 24 and 48 hour after removal of the challenge patch. This study is **Acceptable**.

MRID 47185127: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in mouse OPPTS 870.5395. In this micronucleus test Homobrassinolide Technical did not have micronucleus induction potential in mice after two days of oral dosing up to a level of 2000 mg/kg body weight. **Acceptable** and satisfies the guideline requirement.

MRID 47817702: Teratogenic Evaluation (OPPTS 870.3700). The study showed that treatment with 100 and 1000 mg/kg bw of technical homobrassinolide (HBR) did not result in mortality or overt signs of toxicity for pregnant rats during the observation period. Body weight changes in the groups of test substance treated dams were statistically similar to controls and no significant changes were observed in the weights of ovary and foetuses. The test further showed that there were no significant changes in the uterine weights, as well as, no test related recurrent visceral and skeletal malformations when compared to controls. Based on these findings it appears that HBR was non-teratogenic to Wistar rats at the dose levels of 100 and 1000 mg/kg bw (refer to Appendix A for statistical analysis). This study is **Acceptable.**

Risk Assessment

Brassinosteroids are a group of steroidal plant hormones that were discovered in 1973, when it was shown that pollen from *Barssica napus* could promote stem elongation and cell divisions and that the biologically active molecule was a steroid. Since their discovery, over 70 brassinosteroids have been isolated from plants. The occurrence of these steroids have been demonstrated in various plant parts, such as pollen, flower buds, fruits seeds, vascular cambium, leaves, shoots and roots. Studies on higher plants suggest that these steroids play a critical role in a range of developmental processes (i.e. stem elongation, root growth, floral initiation, etc). The molecular mode of action of brassinosteroids is still being studied. However, the binding of the brassinosteroid molecule to an receptor in the cell plasma membrane appears to activate the kinase domain and subsequent phosphorylation of additional kinases and/or phosphotases (Setta *et al.* 2002).

Homobrassinolide Technical is a technical grade active ingredient/manufacturing use product to be used only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% w/w homobrassinolide (2α, 3α, 22S, 3S, 24S)-2, 3, 22, 23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one). There are no intentionally-added inert ingredients in the product. Impurities in the product are [redacted] The CSF and product label are in agreement regarding the active ingredient content. The beginning materials were described, but the MSDSs submitted were not from the suppliers specified in MRID 47185101. The product is produced using an integrated process, and an adequate discussion of the formation of the impurities was provided. Acceptable results from analysis of five lots of Homobrassinolide Technical were submitted. The certified limits for the active ingredient are within the OPPTS 830.1750 guidelines. The enforcement analytical method is high performance liquid chromatography with ultraviolet detection. The physical/chemical characteristics were adequately presented. Results of acute studies with Homobrassinolide Technical are summarized in Table 2. Based on those studies, the registrant found the test material to be practically non-toxic, with no human/mammalian health problems expected (**Toxicology Category III and IV**).

Human Health

Toxicological Profile for Homobrassinolide is presented in Table 3.0. This data suggests that the compound is in Toxicity Category III - IV for oral and dermal toxicity, Toxicity Category IV for acute inhalation toxicity, Toxicity Category III for primary eye irritation (exposure to eyes results in a mild irritation), and Toxicity Category IV for primary dermal irritation (not a dermal sensitizer). Studies also show that homobrassinolide is not cytotoxic, does not cause chromosomal aberrations, is not a sensitizer or a dermal irritant and is not teratogenic. The request for a waiver for immunotoxicity was deemed acceptable because the 90-day oral toxicity study in rats (MRID 47208906) showed a NOAEL of > 1000 mg/kg/day with no changes in organ weights or differential white blood cell counts. This suggests that homobrassinolide should not cause potential interference with normal immune function.

Dietary Exposure to Humans

The registrant has provided information on the safety of homobrassinolide which covers human and environmental safety. Although the metabolism of brassinosteroids is poorly understood, these plant steroids appear to be metabolized in plants to give the inactive forms, through transformation in the side chain or in the steroid skeleton. Brassinosteroids are present in all plants, resulting in ubiquitous exposure to humans and other organisms through the food chain without causing harm. The endogenous levels are in ppm to ppb levels (i.e. brassinosteroids levels in pollen have measured at 200 ppb). The registrant claims that the small quantity (< 20 g/A) of homobrassinosteroids that is proposed for application as a plant growth stimulant is unlikely to increase levels of brassininosteroids in the treated plants. They reason that the amount of exogenenous brassinosteroid that is applied to a crop plant will be metabolized as the plant grows. Therefore, levels in the human diet are unlikely to be affected. Calculations suggests that human/wildlife exposure to homobrassinosteroids is expected to be low with 20 g/A = 20,000 mg/43,560 ft^2 = 0.459 mg/ft^2. Potential toxicity is low with acute oral toxicity value > 5 g/kg/bw, acute dermal > 2 g/kg/bw, and acute inhalation is 2.2 mg/L. This suggests that risk from exposure is very low at 20 g/A and that even if the entire acre of exposed plants were consumed (unlikely) by an individual, the toxicity from homobrassinosteroid is >10X below the toxicity values noted above. The applicant has requested tolerance exemption for residues of homobrassinolide in or on crops and use sites. The registrant also request waivers for all tests for determining the residues including the analytical method. **Based on the available data, the Agency feels that there are reasonable grounds (as noted above) for tolerance exemption for the proposed uses of homobrassinolide in/on all raw agricultural commodities. The Agency also accepts the registrant's argument that for low homobrassinolide toxicity and exposure to humans/wildlife and feels that exemption from the required tolerance of residues in or on raw commodities is warranted.**

Occupational and Residential Exposure

The product is not intended for homeowner (residential) use and therefore, there will be no residential exposure. The label statement is for manufacturing use only. Directions for use state that the product is intended only for formulation into plant growth regulator end-use products. Each formulator is responsible for obtaining EPA registration of the respective end-use product(s). Although this compound is expected to be used on all crops there are no labels for specific crops.

Drinking Water

It seems unlikely that homobrassinolide concentrations would exceed levels that are currently ubiquitous to plants. Although Fate information is not available, the compound is not soluble in water (water solubility 3.18%), and the **log P_{ow} = 3.96 suggests moderate binding to soil and a low probability of ground water contamination.**

Non-Target Organism Hazard Assessment and Endangered Species Concerns

The proposed uses of homobrassinolide include mostly pre-harvest applications on agricultural crops, and ornamental and forest trees (terrestrial, food crops, greenhouse food and non-food crops, aquatic food and non-food crops). The proposed uses cover all crops (food and non-food), grasses, vines and trees listed under 40CFR Part 158, Appendix A. Ecotoxicity data shows that this compound is slightly toxic to freshwater fish (LC_{50} = 14.38 – 24.56 mg/L) and moderately toxic to aquatic invertebrates (EC_{50} = 8.90 mg/L). Toxicity to mammals is in the slight to practically non-toxic range (LD_{50} >1,000 to 5,000 mg/kg). Since this compound is ubiquitous to plants, and has low toxicity to vertebrates and invertebrates, it can be assumed that there is low to no toxicity to other non-target organisms such as plants, avian, and insects. In evaluating possible impact to aquatic and terrestrial endangered species the Agency extends the above assumptions and data and predicts no direct or indirect impact to endangered species from the use of homobrassinolide. This was determined by employing the Individual Chance Model version 1.1 that uses the probit dose-response curve and the median lethal estimate in predicting effects to an individual. The default slope of 4.5 was used in determining the chance of an individual effect. Using the acute endangered species level of concern (LOC) of 0.05 and the default slope of 4.5 the chance of an individual mortality for aquatic endangered species is ~ 1 in 410,000,000 and for terrestrial endangered species it is ~ 1 in 294,000.

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